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Sara Zaina[1], Giuseppe Mastrangelo[2], Maria Nicoletta Ballarin[3], Luca Scoizzato[4], Giorgio Carradori[5], Ugo Fedeli[6], Silvana Capella[7], Elena Belluso[8]

[1] Department of Cardiac, Thoracic, and Vascular Sciences, Unit of Occupational Medicine, University of Padova, Padova, Italy, zara-83@libero.it

[2] Department of Cardiac, Thoracic, and Vascular Sciences, Unit of Occupational Medicine, University of Padova, Padova, Italy, giuseppe.mastrangelo@unipd.it

[3] Health and Safety at Work Department (SPSAL), Local Health Authority No. 12,Veneto Region, Mestre-Venezia, Italy, nicoletta.ballarin@ulss12.ve.it

[4] Health and Safety at Work Department (SPSAL), Local Health Authority No. 12,Veneto Region, Mestre-Venezia, Italy, luca.scoizzato@gmail.com

[5] Health and Safety at Work Department (SPSAL), Local Health Authority No. 12, Veneto Region, Mestre-Venezia, Italy, sai_giorgio.carradori@ulss12.ve.it

[6] Regional Epidemiology Service, Veneto Region, Padova, Italy, ugo.fedeli@regione.veneto.it

[7] Department of Earth Sciences; and Interdepartmental Center 'G. Scansetti' for Studies on Asbestos and other Toxic Particulates, University of Torino, Torino, Italy, silvana.capella@unito.it

[5] Department of Earth Sciences; and Interdepartmental Center 'G. Scansetti' for Studies on Asbestos and other Toxic Particulates, University of Torino, Torino, Italy, and CNR, Institute of Geosciences and Earth Resurces, Operative Support Unit of Torino, Torino, Italy, elena.belluso@unito.it

Corresponding author: Giuseppe Mastrangelo

ABSTRACT

To assess the validity of the procedure as test of exposure to asbestos, we compared urinary asbestos fibers with occupational and environmental exposure data in a random sample of 48 patients. Occupational and environmental asbestos exposure were estimated on questionnaire, pleural plaques were diagnosed with computed tomography, inorganic fibers and particles were identified by scanning electron microscope with an energy dispersive spectrometry. Few urinary asbestos fibers (in 15% of workers and in 17% of cases with pleural plaques) and high amount of urinary silicate (particularly nonfibrous particles) were detected. Asbestos undergoes dissolution in lung tissues but the secondary minerals are largely unknown. These materials, possibly nonfibrous silicates or metals, could be excreted with urine. Therefore, another study including a control group is warranted to discriminate the occupational origin of minerals in the urine.

Keywords. Asbestos fibers, fibrous silicates, nonfibrous silicates, urine, scanning electron microscope with energy dispersive spectrometry (SEM-EDS)

BACKGROUND

Airborne mineral particles reaching lung alveoli undergo several different processes. First, they can simply remain free in the alveolus (or eventually removed via the mucociliary system). Secondly, they can be partially or completely dissolved since lysosomal fluids, released mainly by alveolar macrophages, have an acidic pH. A third possible fate is to be ingested by macrophages. Macrophages have a diameter of about 10 mm, and are capable of engulfing particles smaller than 5 mm. Larger fibres cannot be completely engulfed, giving rise to the discharge of lysosomal fluids and enzymes. A fourth fate involves migration of mineral particles, either naked or inside macrophages, across the alveolar membrane and into the interstitial lung tissue. They can either remain there or reach the lymphatic system. Particles entering the lymphatic ducts tend to be filtered off at lymph nodes, where they may stay indefinitely. However, some of them pass through the lymph into the blood circulation and can thus reach other organs of the body [1]. In subjects with occupational or domestic/environmental exposure to asbestos, Huang [2] and Molinini [3] reported a long list of studies documenting the presence of asbestos fibers in various organs of the digestive tract (esophagus, stomach, colon, liver), urinary tract (kidney and bladder) and other organs (peritoneum, spleen, ovary). Fiber deposition was found relevant in the kidney and in the liver [4].

Urinary asbestos concentrations were evaluated as indicator of occupational exposure to chrysotile asbestos already in 1985 [5]. The urinary asbestos levels were significantly greater in exposed workers than in the control group; however, the project was not specifically designed to correlate urinary and airborne asbestos concentrations [5]. More recently, the examination of inorganic fibers in urine was used for mapping asbestos environmental exposure in different areas of the Piedmont Region (Susa Valley, Lanzo Valleys and Sesia

Valley) [6]. This study showed that asbestos fibers in the urine can be a good method for quantitative assessment of recent exposure to natural sources of asbestos fibers.

Detection of asbestos in the urine represents the first step in developing a biological indicator of exposure. Since there were no preliminary data, we aimed to investigate in the present study a possible correlation between urinary concentrations and estimates of airborne asbestos fibers concentrations. Scanning electron microscopy (SEM), equipped with an energy dispersive spectrometry (EDS), was selected as the most sensitive technique for qualitative as well as quantitative evaluation of urinary inorganic fibers and particles (both exogenous and endogenous). We then compared the urinary findings with occupational and environmental exposure data in order to assess the validity of this procedure as test of exposure to asbestos.

METHODS

Selection of workers with past asbestos exposure

Among the patients examined from 2000 to 2011 in the Department of Health and Safety at Work (SPISAL, Italian acronym), Local Health Authority No. 12, Veneto Region (Italy), we identified 273 workers with high past asbestos exposure and bilateral pleural plaques or unilateral pleural thickening, diagnosed with low dose computerized tomography (LDCT). Among them, we randomly selected 55 subjects to which we sent a letter of invitation explaining methods and purposes of study. Lastly, we contacted by telephone the 48 accepting patients to make an appointment, remind the collection of urine, and recommend not using talc several days before the medical visit. Among these patients, age (mean±standard deviation) was 70±4.9 years; non-smokers, ex-smokers and smokers constituted, respectively, 58.3% (=28/48), 31.3% (=15/48) and 10.4$ (5/48). All subjects signed an informed consent before collecting urine. Approval by the local ethics committee

was not required since the medical surveillance of workers formerly exposed to asbestos was a mandatory activity (Veneto Region Decree Law n. 5094, Dec 28th 1998; and Veneto Region Directorate Decree No. 48 of 20/12/2006).

Detection of inorganic fibers and particles in urine.

About 60 cc of urine were collected for each subject in containers with screw cap and shipped to the laboratory of the Department of Earth Sciences, University of Torino (Italy).

The urine sample preparation has been carried out using the protocol to detect, identify and quantify inorganic fibers in biological samples by SEM-EDS according to Belluso [7].

Urine samples were prepared according to the main three following steps:

- chemical digestion in sodium hypochlorite 13% was used in order to “digest” organic materials;
- the obtained suspension was filtered on mixed cellulose esters membrane with a diameter of 25 mm and a pore size of 0.45 μm;
- the membrane were dried, attached on the stubs using bi-adhesive scotch and made conductive by carbon sputter coatings.

For each membrane, 800 microscopic fields were observed at 2000 magnification following a standardized procedure [7]. Each inorganic particle and each "breathable fiber" (length > 5 μm, diameter < 3μm and aspect ratio > 3) was measured.

The inorganic fibers and particles recovered were identified by their EDS spectra compared with spectra from a database created by the research team working at the Department of Earth Sciences at the University of Torino. For each sample, the amount of f/ml was calculated.

Assessment of occupational and environmental exposure

Occupational asbestos exposure was estimated with a stepwise procedure that considers initially the materials used (fiber content and friability), then the tasks performed (specified in terms of mechanical stress applied to the material by tools directly used by the worker), and finally the factors modulating exposure (surface of the source, presence of local exhaust systems, etc.). For each aspect, defined as “determinant" of exposure, a score was assigned on the basis of scales defined in tables. The integration of different scores led to a semiquantitative estimate of concentration (i) which, together with percentage of working time spent at that concentration (f) and years of exposure duration (l), allowed to obtain the semi-quantitative estimate of cumulative exposure to asbestos ($i \times f \times l$). If a subject changed job or factory the cumulative exposure was the sum of products ($\Sigma (i \times f \times l)$). The interviewers were trained in the use of the questionnaire in order to minimize the information bias [8]. Length of exposure (years), time elapsed since first and last exposure (years), peak asbestos level (highest asbestos exposure for any job held, fibers/ml) and cumulative asbestos exposure (fibers×years/ml) have been considered in the present study.

Environmental asbestos exposure from birth onward was collected with a questionnaire used at the Department of Earth Sciences, University of Torino. The questions were focused on: characteristics of neighborhood of residence; presence of asbestos-cement roofing in the district (and calendar year of last exposure); existence of nearby industries or railways (and calendar year of last exposure); domestic heating boiler with asbestos insulation (and calendar year of last exposure); non-occupational activities or hobbies involving a possible exposure to asbestos (and calendar year of last exposure). The questionnaire also investigated pets and litters for pet at home, personal use of talc, smoking habits and drinking mineral or tap water.

Statistical analysis

After asbestos ban in Italy in 1992, the only sources involving a possible exposure to asbestos in our subjects were residence (near asbestos-cement roofing or close to industries or railways), domestic heating boiler with asbestos insulation, non-occupational activities or hobbies, recent and constant use of talc and drinking tap water. We coded a dichotomous variable, which was equal to 1 each time that a specific environmental exposure persisted at least 10 years after the end of occupational exposure. We did not considered the old environmental sources of asbestos like, for example, the many reports of having had at home a heating system with asbestos insulation because this exposure ceased long time ago, often before the end of occupational exposure.

The 48 study subjects were broken down by presence/absence of asbestos fibers in urine and according to different aspects of asbestos exposure. The Fisher's exact test was used for frequency variables and Wilcoxon-Mann-Whitney test for interval variables; the p-value for a two-sided test was set at 0.05 for statistical significance.

RESULTS

Historical occupational exposure to asbestos was heavy in all study subjects. The estimates (mean±standard deviations) were: 26.7±6.8 years for length; 245.7±112.5 fiber×years/ml for cumulative exposure; 48.5±6.4 years for time since first exposure; 19.4±6.3 years for time since last exposure; and 87.6±60.6 fibers/ml for peak exposure. Asbestos pleural plaques (diagnosed with LDCT) were 89% (=42/48). Environmental exposure was sporadic. Urinary asbestos fibers were detected in 15% (=7/48) of exposed workers and in 17% (=7/42) of patients with pleural plaques.

In the urine of 48 examined subjects, fibers represented only 7.5% of the total particles (405 fibers/ml against 4970 particles/ml). The detected compounds were grouped according to the chemical content; furthermore, silicates were distinguished according to the usual mineralogical classification [9].

Figure 1 shows the percent distribution of not fibrous compounds. In addition to Ca-oxalates (20.2%) that are organic mineral of probable endogenous origin, eight kinds of inorganic particles were detected: vitreous silicates (14.9%); inosilicates (3.4%); orthosilicates (0.8%); phyllosilicates (29.8%); tectosilicates (14.2%); other silicates (i.e. silicates containing different metals and not included in previous groups, 1.8%); sulphates and phosphates (3.8%); oxides (10.2%); and compound not determined (0.9%). Silicates (excluding vitreous silicates) stand for 50% of total nonfibrous particles detected in the urine, and phyllosilicates were the main single component (29.8%).

Figure 2 shows the percent distribution of fibrous compounds. In addition to Ca-oxalates (5.7%) that were also found as fibers, ten kinds of inorganic fibers were identified: vitreous silicates (20.8%); inosilicates (except asbestos tremolite, 3.8%); phyllosilicates (except chrysotile-antigorite, 17.0%); tectosilicates (18.9%), other silicates (i.e. silicates containing different metals and not included in previous groups, 1.9%); sulphates and phosphates (5.7%); oxides (7.6%), aluminum (3.8%); tremolite-actinolite asbestos (7.6%) and chrysotile-antigorite (7.6%). Tremolite-actinolite asbestos fibers were grouped together because their chemical characterization cannot be determined by qualitative SEM-EDS analysis. Likewise, because of the difficulty in distinguishing chrysotile (asbestos) and antigorite (non asbestos) using this technique, chrysotile and antigorite were considered as a sole group, named chrysotile-antigorite group and considered asbestos in this study [10]. Silicates (excluding

vitreous silicates) stand for 57% of total inorganic fibers detected in the urine; again, phyllosilicates (including chrysotile-antigorite) were the main single component (24.6%).

Asbestos fibers were found in only seven subjects: chrysotile-antigorite in four and tremolite-actinolite asbestos in three workers. In these seven subjects (all with bilateral pleural plaques) table 1 shows age, smoking habit, chemical composition and size of urinary asbestos fibers detected with SEM-EDS, along with occupational asbestos exposure data (length, cumulative exposure, time elapsed since first exposure, time elapsed since last exposure, and the highest peak asbestos level for any job held) and environmental asbestos exposure characteristics. It can be seen that occupational exposure was massive in every subjects, while environmental exposures were uncommon.

Table 2 shows the same exposure variables of table 1 in subjects without (number = 41) and, separately, with (number = 7) asbestos fibers in urine. We reported mean and standard deviation of interval variables in table 2a, or number and percentage of frequency variables in table 2b. No statistical test provided a p-value close to the threshold of statistical significance ($p < 0.05$). There was no relationship between any aspect of asbestos exposure and asbestos fibers in urine.

DISCUSSION

The idea for the present study came from the findings of inorganic fibers in urine of subjects living in different areas of the Piedmont Region, Italy, with outcropping rocks containing asbestos [6]. The airborne concentration of asbestos fibers, not measured in these environments, could probably be close to the 0.007 f/ml measured in outdoor air in a rural village of a region of Turkey with natural sources (rocks containing tremolite) of asbestos [11]. On the other hand, the threshold limit values for asbestos occupational exposure

(suggested by the American Conference of Governmental Industrial Hygienists) were 12 f/ml in 1968-1969, 5 f/ml from 1970 to 1978, and 2 f/ml from 1978 to 1990 [12]. During the 24-year history of regulation of exposure to asbestos, the time-weighted average concentration was ((12×2) + (5×9) + (2×13))/24, equal to 7.7 f/ml [12]. During the same period, the average concentration of asbestos in the workplace was equal 7.2 f/ml in 772 past exposed workers in Veneto region of Italy [13]. Since in these subjects the correlations between historical exposure and benign asbestos-related diseases were consistent with those of earlier studies carried out with conventional chest x-rays, the estimates of occupational exposure to asbestos were accurate and precise [13]. Occupational exposure was about three orders of magnitude higher than exposure from natural sources. We therefore presumed that asbestos fibers could be present in urine despite workers were no longer exposed after 1992 (asbestos ban in Italy). It is known that chrysotile dissolves fairly rapidly in lung tissue (half-life of approximately 6 months – some papers suggest that the time period may be even shorter – which is not surprising given that only amphiboles are acid-resistant, and that the pH inside a macrophage lysosome, is up to 4. The dissolution of chrysotile in lung tissue has been confirmed experimentally [14,15]. The dissolution of pulmonary amphiboles, however, is slower. It has been calculated that in humans the elimination of crocidolite fibers is 10 to 15% per year, which means that every 7-10 years half of the contents of fibers accumulated in the lung will be removed [16]. Therefore, amphiboles may still be present in lung and other organs of the most exposed workers. In view of all the above, we attempted to establish urinary analysis of fibers as a reliable tool of biomonitoring for past heavy asbestos exposure. The present study found urinary asbestos fibers in only 15% (=7/48) of highly exposed workers demonstrating that SEM-EDS mineralogical analysis of urine could not be a reliable test for determining how much asbestos might be in the lungs. Only 7 (17%) out of 42 cases with bilateral pleural plaques were found to have asbestos fibers in urine indicating that this test cannot predict the

risk of asbestos-related disease. Therefore, urine test could not be an indicator of past exposure nor effect. We cannot confirm earlier evidence of urinary asbestos fibers in subjects exposed to airborne asbestos from environmental sources [6], or in subjects drinking water contaminated with asbestos [3]. According to the "Toxicological profile for asbestos", published in 2001 by Agency for Toxic Substances and Disease Registry, only a tiny fraction of inhaled or ingested fibers is excreted in the urine, the quantitative relationship between exposure and urinary fiber concentration appears quite variable and, lastly, urinary levels presumably are mainly a reflection only of recent exposures and not the cumulative tissue burden [17]. These statements were not referenced, however, and this fact led to the present study.

In Biancavilla area (Catania, Sicily Region), fibers of amphibole fluoro-edenite were released by autochthon lava rocks, also used as building material until few years ago. Mineral fibers in urine samples were examined by SEM-EDS in 15 people living in Biancavilla (Group 1) and in a control group of 20 people living in Asti, Piedmont Region (Group 2). In both groups, no subject was occupationally exposed to asbestos. Fibrous silicates (mainly phyllosilicates and inosilicates) were detected in 13/15 subjects in Group 1 and in 1/20 individuals in Group 2. The authors concluded that the presence of silicate fibers in urine was correlated to environmental exposure to airborne particulate [18].

In regulation and public perception there is the idea that asbestos is one specific substance occurring in six different forms (chrysotile, amosite, crocidolite, anthophyllite, tremolite, and actinolite). This could be incorrect according to Guidotti [19] and Baumann [20]. Asbestos is not a proper mineralogical or geochemical term. The defining characteristics of asbestos are simply that it is fibrous, silicate, and has been traditionally called "asbestos" in commerce, history, and convention [19]. There are many other fibrous silicates, besides the six that are

arbitrarily called “asbestos”, that often have effects on lung tissue very similar to asbestos and in some cases show even greater potency for fibrogenesis and cancer initiation, as noted for fibrous erionite and Libby material [19]. In all, there are approximately 70 such “nonasbestos” fibrous silicates among natural minerals, some rare and some quite common, and increasingly more synthetic silicates. Sometimes, these asbestiform minerals appear as hazards in construction, mining, and industrial applications [19]. Baumann [20] and Guidotti [19] proposed that all fibrous minerals should be handled as potentially pathogenic until they are proven safe.

In the present study, high amounts of nonvitreous silicate fibers (phyllosilicates in particular) were detected in the urines of asbestos workers. Inclusion of a control group in a future study could allow discriminating the occupational rather than environmental source, because inorganic fibers are widespread in many industrial products and in outdoor air, where they are released by anthropogenic works and natural rocks and dispersed by atmospheric agents [21].

Longer asbestos fibers that are retained in the lung undergo three simultaneous clearance processes: alveolar macrophage-mediated clearance; dissolution of fibers in the lung fluid; and breakage of long fibers into shorter fibers. After phagocytosis by macrophages, fibers are coated with a matrix containing protein, mucopolysaccharides and iron. The coat may be variably segmented into spherical or rectangular units spaced along the fiber, and the ends are frequently knobbed. Finally, the cell dies and asbestos fiber is released into pulmonary parenchyma as asbestos body [22]. The latter structure might be less able than naked fiber to perforate the lining of lung and reach urinary organs. This could explain why analyses evidence asbestos fibers in urine only in recent exposures. The dissolution of asbestos in lung fluid is a complex process related to the effects of H+ breaking Si-O-Mg, in conjunction with water producing Si-OH and hydrated Mg2+ [23]. The dissolution reactions might have the

potential to modify the fibers in secondary minerals that could be excreted with urine. In the present study, the concentration of nonvitreous nonfibrous silicates (phyllosilicates in particular) detected in the urines of asbestos workers was 10 times higher than that of the fibrous counterparts. In the aim to identify a compound related to the dissolution of the fibers in tissue fluids, a future study should include a control group that allows discriminating the occupational rather than environmental source of silicate particles detected in the urine with electron microscopy.

CONCLUSIONS

SEM-EDS mineralogical analysis of urine could not be a reliable indicator of past asbestos exposure nor asbestos effect. The high amount of silicates found in urine deserves further investigations in the hypothesis that some non-asbestos silicate compounds could originate from occupational exposure.

ABBREVIATIONS

SEM: Scanning electron microscope; EDS: Energy dispersive spectrometry; LDCT: low dose computerized tomography.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SZ and GM conceived the research idea. SZ and EB designed the experiment. SZ, EB and SC performed all experiment procedures. MNB, GC and LS examined workers estimating the

occupational and environmental asbestos exposure. GM and UF performed the statistical analysis. UF, EB and GM drafted the manuscript.

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Table 1. Past exposed workers with urinary asbestos fibers: characteristics of subjects, exposures and fibers.

			Worker #1	Worker #2	Worker #3	Worker #4	Worker #5	Worker #6	Worker #7
Age (years)			72	64	74	69	70	77	67
Smoking			Ex-smoker	Ex-smoker	Ex-smoker	Ex-smoker	Smoker	Non-smoker	Non smoker
Occupation	Length (years)		28	26	26	24	23	39	24
	Cumulative exposure (ff×years/cc)		157.15	399.31	348.30	327.11	325.59	182.41	132.98
	Peak exposure (ff/cc)		13.5	135	135	135	135	13.5	13.5
	Time since first exposure		49	45	56	48	42	57	44
	Time since last exposure		19	15	24	21	17	18	20
Environmental exp.	Cement-asbestos roofing nearby		1						
	Industries/railways nearby				1				
	Activities/hobbies			1					
	Pet at home			1	1				
	Use of talc				1				
	Drinking tap water			1	1	1	1	1	
Urinary asbestos fibers	Chrysotile-antigorite (fiber size)	Diameters (µm)	6.87×2.09		7.34×2.05	6.74×1.83			8.22×1.72
		Aspect ratio	3.29		3.59	3.68			4.78
		Diameters (µm)				2.37×0.7			
		Aspect ratio				3.39			
	Asbestos Tremolite (fiber size)	Diameters (µm)		7.43×1.42			5.24×1.10	10.6×1.10	
		Aspect ratio		5.23			4.76	9.64	
		Diameters (µm)		5.92×1.58					
		Aspect ratio		3.75					

Table 2. Mean and standard deviation (sd) for interval variables (table 2a), number of subjects (N) and percent (%) for frequency variables (table 2b) and two-sided p-value of the statistical test in the two groups of past asbestos workers: without and with asbestos fibers in urine.

	Asbestos fibers in urine				p-value
	Without (N = 41)		With (N = 7)		
(table 2a)	mean	sd	mean	sd	
Age (years)	68.8	5.0	69.0	4.2	0.965
Length of exposure (years)	26.6	7.1	27.1	5.5	0.649
Cumulative exposure ((ff×years)/cc)	242.0	114.3	267.5	106.7	0.609
Time since first exposure (years)	48.3	6.7	48.7	5.8	0.759
Time since last exposure (years)	19.5	6.8	19.1	2.9	0.883
Peak exposure (ff/cc)	87.7	59.9	82.9	64.9	0.811
(table 2b)	N	%	N	%	
Smokers and ex-smokers	27	65.9	5	71.4	1.000
Drinking tap water	27	65.9	5	71.4	1.000
Pet at home	13	31.7	2	28.6	1.000
Cement-asbestos roofing nearby	7	17.1	1	14.3	1.000
Industries/railways nearby	12	29.3	1	14.3	0.656
Leisure time activities and hobbies	0	0.0	1	14.3	0.146

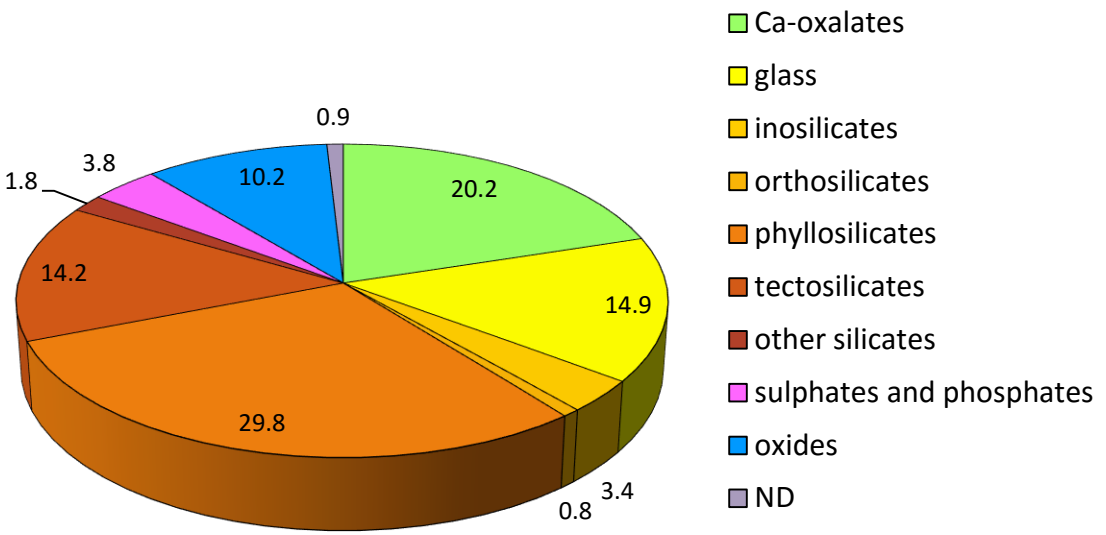

Figure 1. Percent distribution of not fibrous compounds found in the urine of 48 past asbestos workers. ND = not determined

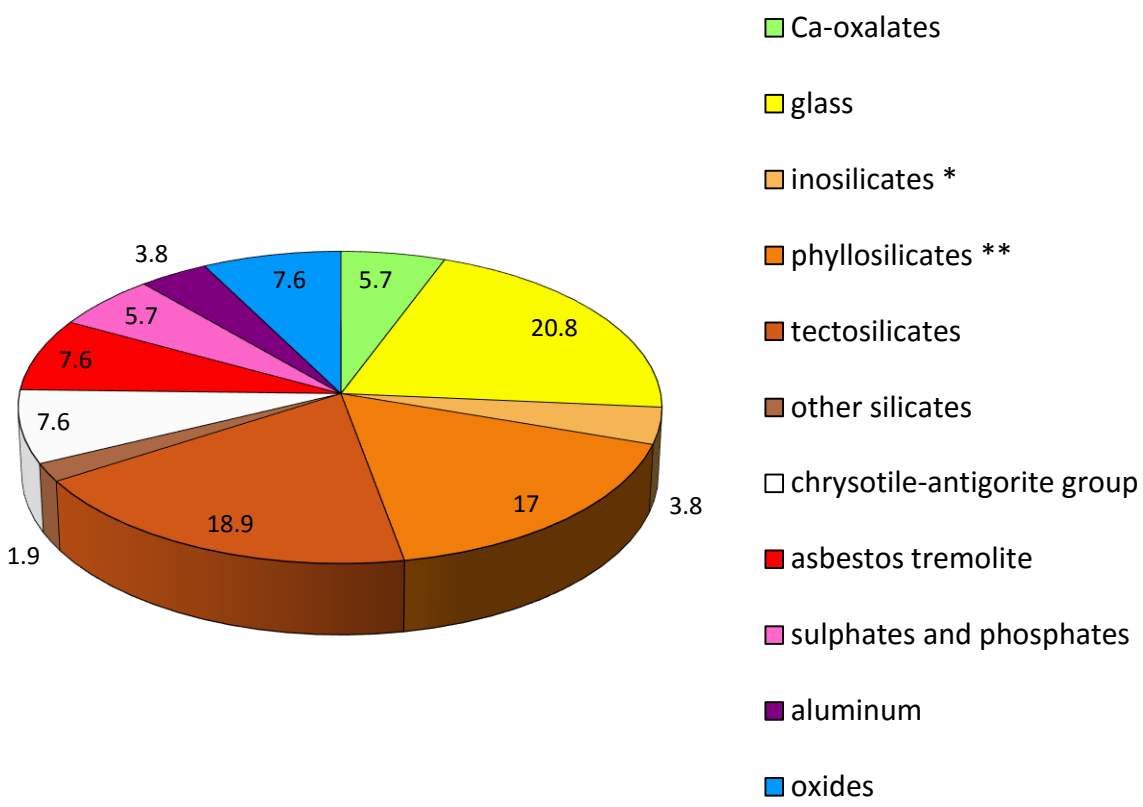

Figure 2. Percent distribution of fibrous compounds found in the urine of 48 past asbestos workers.
* except asbestos tremolite; **except chrysotile-antigorite group